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Ultrastructure of the Pyrenoid in the Family Cladophoraceae (Cladophorales, Chlorophyta)

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The pyrenoid structure was examined with TEM in 14 Japanese species belonging to four genera of the family Cladophoraceae. Four types of pyrenoids were recognized; i.e. bilenticular, zonal, simple polypyramidal and complex polypyramidal types. The pyrenoid matrix of the bilenticular pyrenoid is traversed by a single thylakoid band, the matrix being composed of a pair of hemispheres and surrounded by two starch sheaths. The pyrenoid matrix of the zonal type is divided by parallel thylakoid bands, and is surrounded by more than three starch sheaths. The pyrenoid matrix of the simple polypyramidal type is traversed by three or more thylakoid bands, four or more pieces of starch sheaths or grains covering the surface of the pyrenoid matrix. The pyrenoid matrix of the complex polypyramidal type is divided by intrusions of many sinuous thylakoid bands, and is surrounded by many small starch sheaths or grains. Three or four types of pyrenoid were observed in the genera *Chaetomorpha*, *Cladophora* and *Rhizoclonium*.

Until recently cladophoracean algae were considered to possess hemispherical bilenticular pyrenoids in which the pyrenoid matrix was traversed by a single thylakoid band and surrounded by a pair of starch sheaths (Gibbs 1962, Hori and Ueda 1967, Phillips 1990). Wang (1989) and Miyaji (1995), however, reported that some species of Cladophoraceae have polypyramidal pyrenoids, the matrix of which is traversed by many thylakoid bands and surrounded by many pieces of starch sheaths or grains. The present study was carried out to examine ultrastructure of the pyrenoid of Cladophoraceae.

Materials and Methods

Fourteen Japanese species belonging to four genera of Cladophoraceae were examined. The species, localities and dates of collection are given in Table 1. Most materials were fixed one or two days after collection, while others were fixed a few weeks after collection. All of the marine species were fixed with 2% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.1) with 0.25 M sodium saccharose for 1 hr. For fresh water species, the fixation was made with a solution of 2% glutaraladehyde in 0.1 M sodium phosphate buffer (pH 7.1) for 1 hr. Postfixation was made with 2% osmium tetroxide in 0.1 M sodium phosphate buffer for

Table 1. Species examined in the present study

Species	Locality	Date of collection
Chaetomorpha antennina	Shirahama, Shimoda City, Shizuoka Pref.	May 16, 1996
Ch. basiretrorsa	Shirahama, Shimoda City, Shizuoka Pref.	May 16, 1996
Ch. brachygona	Uranohama, Yamada-cho, Iwate Pref.	Aug. 12, 1996
Ch. crassa	Toji, Shimoda City, Shizuoka Pref.	May 17, 1996
Ch. gracilis	Banda, Tateyama City, Chiba Pref.	June 1, 1996
Ch. moniligera	Uranohama, Yamada-cho, Iwate Pref.	Aug. 12, 1996
Ch. spiralis	Shirahama, Shimoda City, Shizuoka Pref.	May 16, 1996
*Cladophora aegagropila**	Lake Saiko, Ashiwada-mura, Yamanashi Pref.	Oct. 9, 1994
Cl. catenata	Hachijojima Isl., Tokyo Metropolitan	Apr. 27, 1996
Cl. conchopheria	Okinoshima, Tateyama City, Chiba Pref.	June 4, 1996
Cl. ohkuboana	Shirahama, Simoda City, Shizuoka Pref.	May 16, 1996
Cl. stimpsonii	Uranohama, Yamada-cho, Iwate Pref.	Aug. 12, 1996
*Rhizoclonium riparium**	Oiso, Naka-gun, Kanagawa Pref.	June 12, 1996
*Pithophora mooreana**	Nankoku City, Kochi Pref.	Oct. 18, 1996

*freshwater species

1 hr. After the postfixation, the material was transferred into 0.5% uranyl acetate solution and kept for 1 hr. The specimens were dehydrated in a graded acetone series up to 100% acetone and were then transferred into 100% propylene oxide. The materials were then embedded with Spurr's low-viscosity embedding medium (Spurr 1969). Polymerization was carried out at 60°C for 24 hr. The polymerized material was sectioned with a diamond knife on a Sorvall MT2-B or a LKB 2088 ultramicrotome. The section was stained with uranyl acetate and lead citrate, and examined with a Hitachi H-7000 or a Jeol 2000EXII electron microscope.

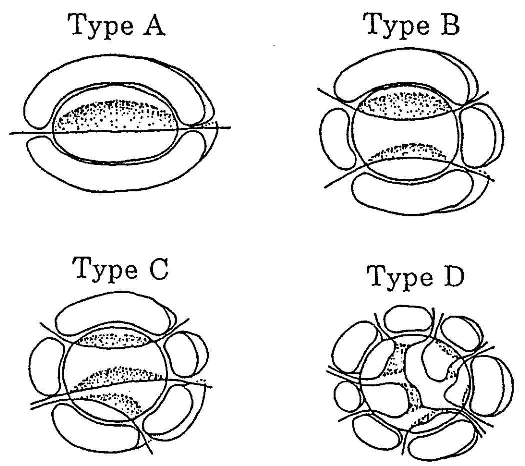


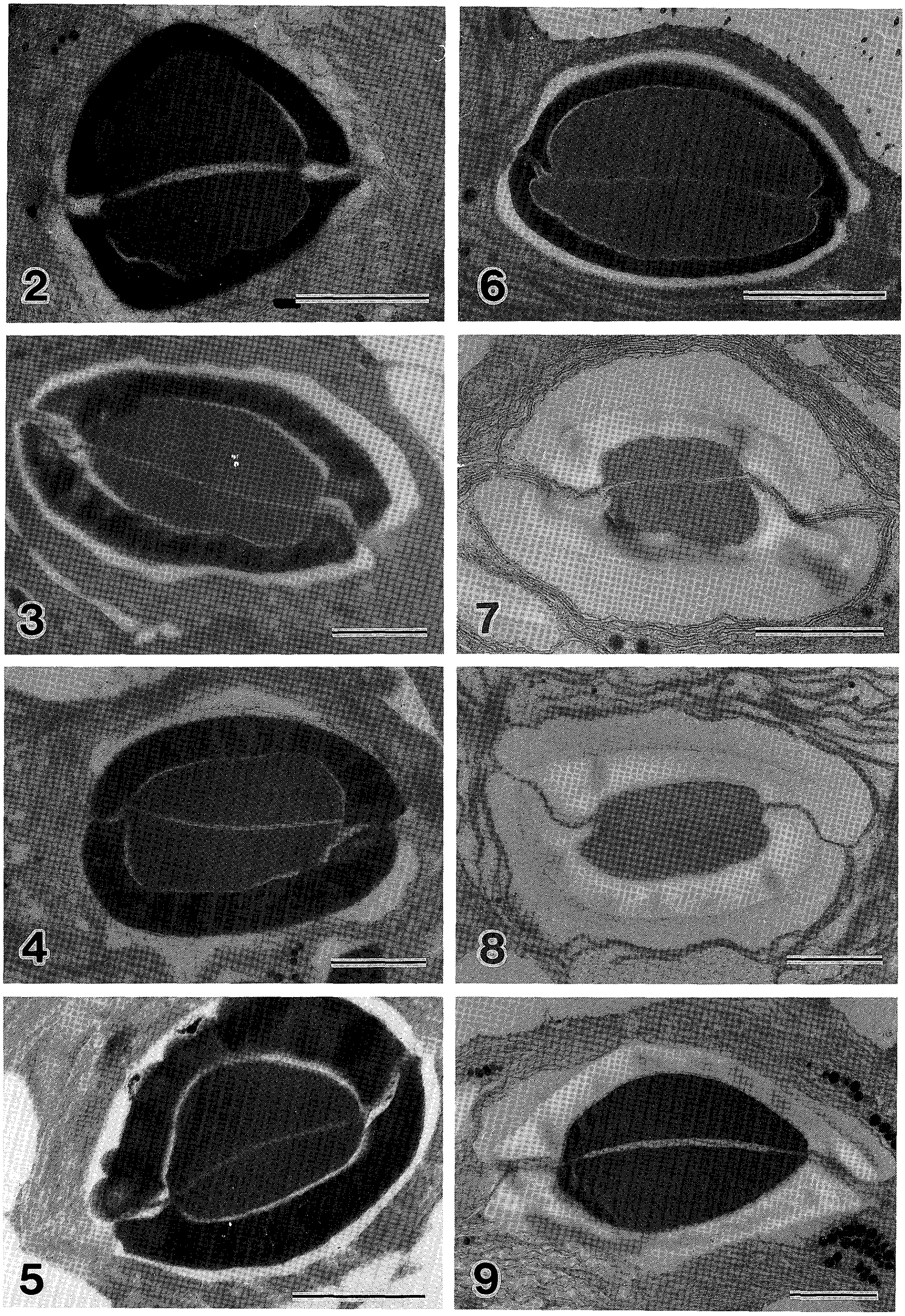
Fig. 1. Schematic drawings of the four types of pyrenoids.

Observations

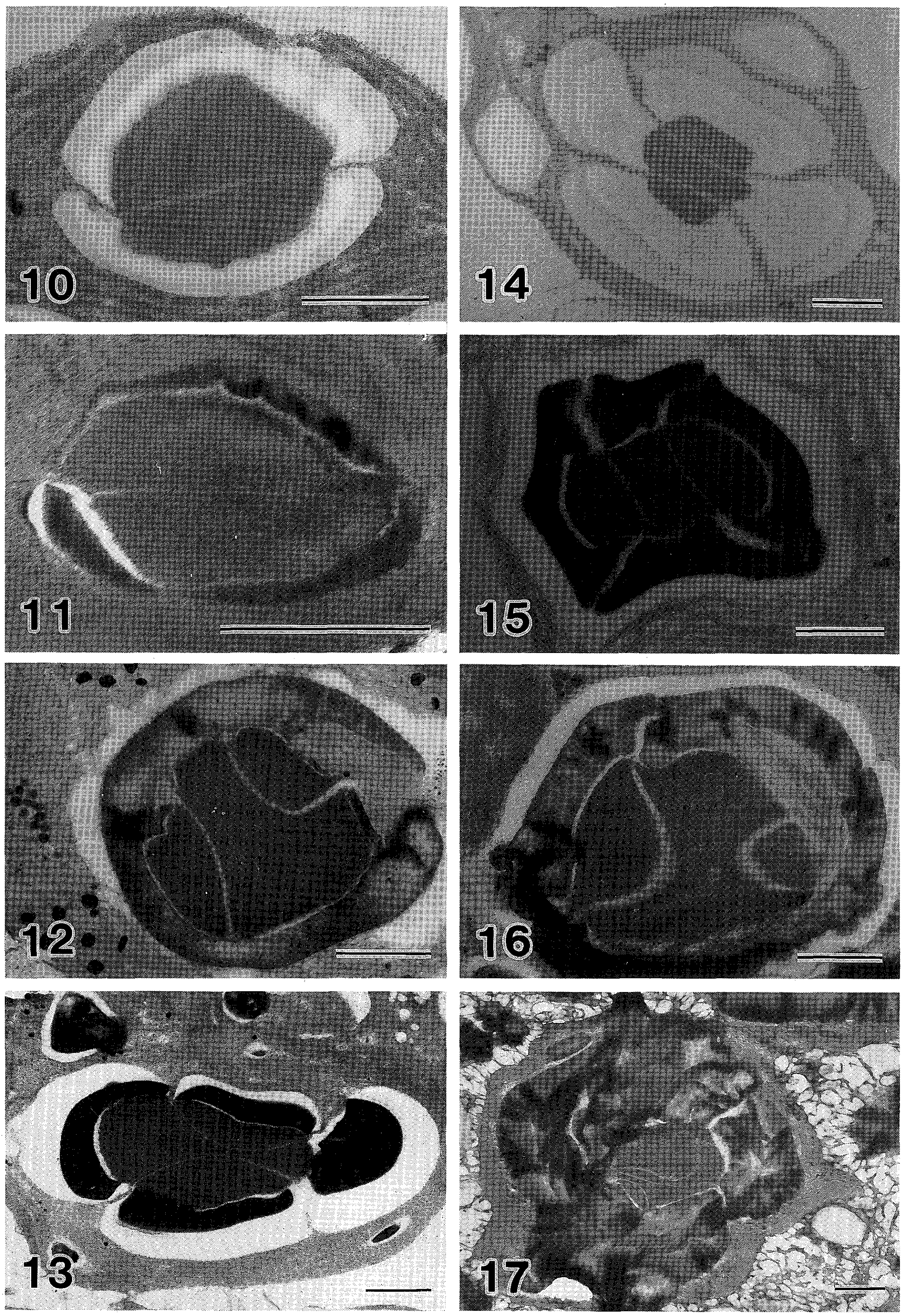
From observations of the structure of pyrenoid matrix, the shape of the associated starch sheaths or grains and the number of intrapyrenoidal thylakoid bands, the four types of pyrenoids (Types A, B, C and D) were recognized in the present materials. These types are schematically shown in Figure 1.

1) Type A (Figs. 2–11)

In this type, the pyrenoid is composed of a matrix traversed by a single thylakoid band which is continuous with the chloroplast thylakoid. The pyrenoid matrix is surrounded by two cup-shaped starch sheaths. This is the most typical pyrenoid in Cladophoraceae and corresponds to the bilenticular pyrenoid defined by Chadefaud (1941). Hori and Ueda (1967) referred to this type of pyrenoid as the "*Cladophora*-type". This type of pyrenoid was



Figs. 2–9. Type A pyrenoids. Fig. 2. *Chaetomorpha antennina*. Fig. 3. *Ch. basiretrorsa*. Fig. 4. *Ch. brachygona*. Fig. 5. *Ch. crassa*. Fig. 6. *Ch. gracilis*. Fig. 7. *Ch. moniligera*. Fig. 8. *Ch. spiralis*. Fig. 9. *Cladophora ohkuboana*. Scale bars = 1 μm.



Figs. 10–11. Type A pyrenoids. Fig. 10. *Cladophora stimpsonii.* Fig. 11. *Rhizoclonium riparium.* Figs. 12–16. Type B pyrenoids. Fig. 12. *Ch. antennina.* Fig. 13. *Ch. brachygona.* Fig. 14. *Ch. moniligera.* Fig. 15. *Ch. spiralis.* Fig. 16. *Cl. ohkuboana.* Fig. 17. Type C pyrenoid.*Cl. aegagropila.* Scale bar = 1 μm.

observed in *Chaetomorpha antennina* (Bory) Kütz. (Fig. 2), *Ch. basiretrorsa* Setch. (Fig. 3), *Ch. brachygona* Harv. (Fig. 4), *Ch. crassa* (C.Agardh) Kütz. (Fig. 5), *Ch. gracilis* Kütz. (Fig. 6), *Ch. moniligera* Kjellm. (Fig. 7), *Ch. spiralis* Okamura (Fig. 8), *Cladophora ohkuboana* Holmes (Fig. 9), *Cl. stimpsonii* Harv. (Fig. 10) and *Rhizoclonium riparium* (Roth) Kütz. ex Harv. (Fig. 11) in the present study. With an exception of *R. riparium*, all of them are marine species.

This type of pyrenoid has already been reported for *Chaetomorpha brachygona* (Chan et al. 1978); *Ch. crassa*, *Cl. wrightiana* Harv. *Rhizoclonium tortuosum* (Dillwyn) Kütz. (Hori and Ueda 1967); *Ch. linum* (Müll.) Kütz. (Gibbs 1962); *Ch. spiralis* (Hirayama and Hori 1984); *Cladophora albida* (Nees) Kütz., *Cl. fascicularis* (Mertens ex C.Agardh) Kütz., *Cl. opaca* Sakai, *Cl. rudolphiana* (C.Agardh) Kütz., *Cl. sakaii* Abbott (Wang 1989); *Cl. flexuosa* (Müller) Kütz. (Scott and Bullock 1976); *Cl. fracta* (Müll. ex Vahl) Kütz. (Strugger and Peveling 1961) and *Cl. glomerata* (L.) Kütz. (McDonald and Pickett-Heaps 1976) (Table 2).

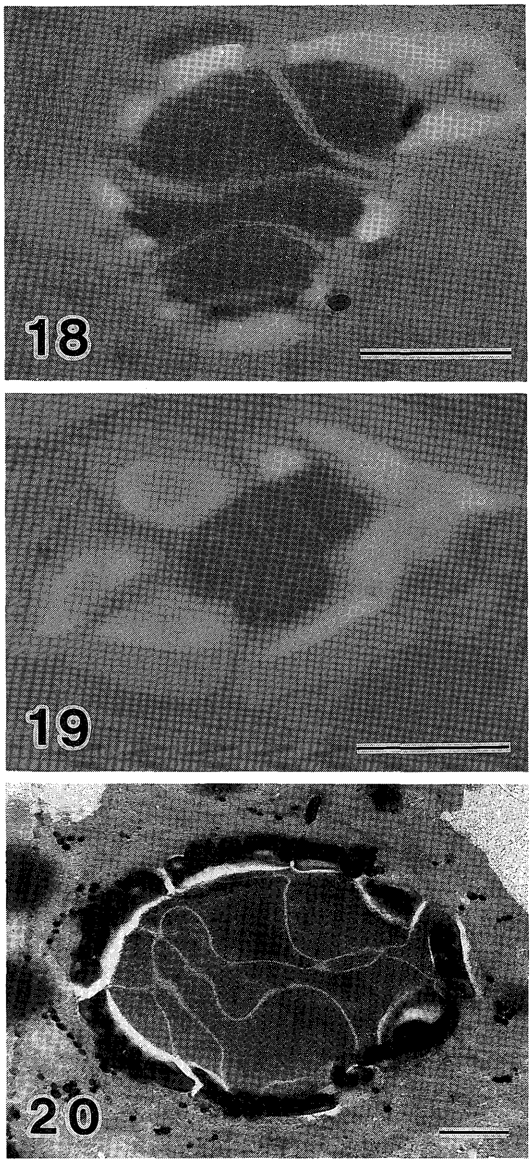
2) Type B (Figs. 12–16)

In this type, the pyrenoid matrix is divided by parallel thylakoid bands, and is surrounded by more than three starch sheaths. This type of pyrenoid was always observed together with Type A pyrenoids but was only rarely observed in cells of the present materials, as *Chaetomorpha antennina* (Fig. 12), *Ch. brachygona* (Fig. 13), *Ch. moniligera* (Fig. 14), *Ch. spiralis* (Fig. 15) and *Cladophora ohkuboana* (Fig. 16), all of which are marine species.

This type of pyrenoid was also reported for *Ch. linum* (Gibbs 1962) and *Rhizoclonium tortuosum* (Hori and Ueda 1967) (Table 2). Even though Chan et al. (1978) described that *Ch. brachygona* has only one type of pyrenoid in which the matrix is divided into two pieces, in the present study this species was found to have two types of pyrenoids (Types A and B).

3) Type C (Figs. 17–19)

In this type, the pyrenoid matrix is traversed by three or more thylakoid bands and four or more pieces of starch sheaths or grains cover the surface of the pyrenoid matrix. This type of



Figs. 18–19. Type C pyrenoids. Fig. 18. *Cladophora catenata*. Fig. 19. *Pithophora mooreana*. Fig. 20. Type D pyrenoid. *Cl. conchopheria*. Scale bar = 1 μm.

pyrenoid was observed in *Cladophora aegagropila* (L.) Rabenh. (Fig. 17), *Cl. catenata* (L.) Kütz. (Fig. 18) and *Pithophora mooreana* Collins (Fig. 19) in the present study.

This type of pyrenoid has already been reported for *Cl. aegagropila* (Miyaji 1995) and *Rhizoclonium* sp. (Miyaji unpublished) (Table 2).

4) Type D (Fig. 20)

In this type, the pyrenoid matrix is divided by intrusions of many sinuous thylakoid bands adjoined with chloroplast thylakoids, and is surrounded by many starch sheaths or grains. Thus, the pyrenoid structure of this type has a more complex appearance as compared with that of Type C. This pyrenoid type was observed in *Cladophora conchopheria* Sakai (Fig. 20) in the present study.

The same type of pyrenoid has already been reported for *C. conchopheria* (Wang 1989) and *Chaetomorpha okamurae* Ueda (Miyaji 1995) (Table 2).

Types A, C and D were observed respectively in different species, whereas Type B was observed always together with Type A. Consequently the 14 species examined in the present study can be classified into four groups

Table 2. Distribution of the pyrenoid types (A, B, C and D) among 27 species of the family Cladophoraceae

Species	Pyrenoid types A	B	C	D	References
Chaetomorpha antennina	●	●			present study
Ch. basiretrorsa	●				present study
Ch. brachygona	●	●			Chan et al. 1978, present study
Ch. crassa	●				Hori & Ueda 1967, present study
Ch. gracilis	●				present study
Ch. linum	●	●			Gibbs 1962
Ch. moniligera	●	●			present study
Ch. spiralis	●	●			Hirayama & Hori 1964, present study
*Ch. okamurae**				●	Miyaji 1995
*Cladophora aegagropila**			●		Miyaji 1995, present study
Cl. albida	●				Wang 1989
Cl. catenata			●		present study
Cl. conchopheria				●	Wang 1989, present study
Cl. fasciculais	●				Wang 1989
Cl. flexuosa	●				Scott & Bullock 1976
*Cl. fracta**	●				Strugger & Peveling 1961
*Cl. glomerata**	●				McDonald & Pickett-Heaps 1976
Cl. ohkuboana	●	●			present study
Cl. opaca	●				Wang 1989
Cl. rudolphiana	●				Wang 1989
Cl. sakaii	●				Wang 1989
Cl. stimpsonii	●				present study
Cl. wrightiana	●				Hori & Ueda 1967
*Rhizoclonium riparium**	●				present study
R. tortuosum			●		Hori & Ueda 1967
R. sp.	●	●			Miyaji (unpublished)
*Pithophora mooreana**			●		present study

*freshwater species

according to the pyrenoid types; the species with Type A, the species with Types A and B, the species with Type C and the species with Type D (Table 2).

Discussion

From observations of the ultrastructure of pyrenoids in relation with intrapyrenoidal thylakoid bands and associated starch sheaths or grains in Cladophoraceae, four types of pyrenoids were distinguished (Table 2). They are bilenticular (Type A), zonal (Type B), simple polypyramidal (Type C) and complex polypyramidal (Type D) pyrenoids.

In the green algae other than Cladophoraceae, there are two genera, *Monostroma* and *Ulothrix*, which were reported to contain pyrenoids similar to those of Cladophoraceae (Hori 1973, Lokhorst and Star 1980). Hori (1973) reported irregularly shaped pyrenoids in *Monostroma fuscum* var. *splendens* (Rupr.) Rosenv. [= *Ulvaria obscura* (Kütz.) Gayral var. *blyttii* (Aresch.) Bliding] and considered that this type of pyrenoid may represent a developmental stage. In the present study, however, it is considered that Type B is intermediate between Type A and Type C.

As for the genus *Pithophora*, the present study is the first one reporting the ultrastructure of the pyrenoid. The pyrenoid of *P. mooreana* was Type C.

Wang (1989) emphasized that the taxonomic position of *Cladophora conchopheria* should be reconsidered since its pyrenoid is polypyramidal. However, it is confirmed in the present study that there are four types of pyrenoids in Cladophoraceae and that three or four different types of pyrenoids are contained within one genus, e.g., three types in *Chaetomorpha*, four types in *Cladophora* and three types in *Rhizoclonium*.

Chadefaud (1941) examined the pyrenoid structure of various green algae by light microscopy, and classified it into five types. He recognized the differences between that of *Oedogonium* and *Platymonas* [= *Tetraselmis*] and referred to them as globulous and umbilical polypyramidal types, respectively. Judging from his schematic drawings, our two types of polypyramidal pyrenoid, which are simple and complex, do not correspond to his two types in construction. However, the zonal type in the present study is probably similar to his zonal type, which Chadefaud observed and drew for *Hormidium* [= *Klebsormidium*].

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松山和世[a]，松岡孝典[b]，宮地和幸[c]，田中次郎[a]，有賀祐勝[a]：**シオグサ科藻類（緑藻シオグサ目）のピレノイドの微細構造**

日本産シオグサ科藻類4属14種のピレノイドの微細構造について透過型電子顕微鏡による観察を行った．シオグサ科藻類のピレノイドは二裂型(チラコイドが1枚貫入した基質を2つのデンプン鞘が囲む)，二裂型＋多層型(平行に複数のチラコイドが貫入した基質を複数のデンプン鞘が囲む)，単純多裂型（複数のチラコイドが貫入した基質を複数のデンプン鞘が囲む)，複雑多裂型(湾曲した複数のチラコイドが複雑に貫入した基質を複数のデンプン鞘が囲む)の4型があった．ジュズモ属，シオグサ属，ネダシグサ属内には種により3～4型のピレノイドが観察された．

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